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Juvenile stress leads to long-term immunological metaplasticity-like effects on inflammatory responses in adulthood

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ABSTRACT

Previous studies indicate that individuals exposed to stress in juvenility are more prone to suffer from stress-related psychopathologies in adulthood. Evidence suggests that exposure to enriched environment (EE) conditions alleviates juvenile stress (JVS) effects. Exposure to stress has been found to affect immune responses to challenges, but whether JVS has long-term effects on inflammatory processes remains unclear. Here, we examined the impact of JVS on inflammatory processes in adulthood, and the effects of exposure to EE conditions. Adult rats exposed to JVS showed elevated levels of blood monocytes after induction of peritoneal inflammation. This was associated with higher concentration of blood chemokine ligand type 2 (CCL2), but lower levels of its receptor, chemokine receptor type 2 (CCR2) on these monocytes, indicating reduced ability of these monocytes to be recruited to the inflammatory site. In accordance, JVS led to reduced levels of recruited macrophages at the peritoneal cavity, as well as a reduced activation ratio for the release of peritoneal interleukin-10 (IL-10) by lipopolysaccharide (LPS) activation. EE conditions, which fully reversed the anxiety-like behavior resulting from exposure to JVS, did not reverse JVS-induced alterations in blood concentration of monocytes or peritoneal macrophages, but affected IL-10 activation ratio. This effect was associated with a compensatory elevation of the peritoneal CCL2-CCR2 axis. Our results demonstrate long-term metaplasticity-like effects of JVS, which alter inflammatory processes in response to immune challenges in adulthood. Our results also raise the possibility that EE does not simply reverse the effects of JVS but rather indirectly modulates its impact.

1. Introduction

The concept of metaplasticity has initially referred to changes that modify the properties of synaptic plasticity due to a priming or preconditioning event, focusing on the synaptic and cellular level (Abraham & Bear, 1996). It has later become apparent that the term could also be useful to describe plasticity changes on a more global level, including environmental stressors as priming events and altered behavior as outcome measures, referred to as "behavioral metaplasticity" (Schmidt, Abraham, Maroun, Stork, & Richter-Levin, 2013). Such behavioral metaplasticity has been shown to be induced by exposure to stress in juvenility, the post-weaning pre-puberty/juvenile stage which holds close resemblance to human late childhood. Specifically, exposure to stress at this stage of life can alter behavioral responses to challenges in adulthood, such as increased anxiety, impaired learning, and poor coping skills (Avital & Richter- Levin, 2005; Avitsur, Levy, Goren, & Grinshpahet, 2015; Brydges, Hall, Nicolson, Holmes, & Hall, 2012; Tsoory & Richter-Levin, 2006; Yee, Schwarting, Fuchs, & Wöhr, 2012), as well as lead to neurophysiological, molecular, and biochemical alterations associated with neural plasticity (Bazak et al., 2009; Brydges, Jin et al., 2014; Brydges, Seckl et al., 2014; Cohen et al., 2007; Grigoryan, Ardi, Albrecht, Richter-Levin, & Segal, 2015; Jacobson-Pick & Richter-Levin, 2012; Tsoory, Guterman, & Richter-Levin, 2008; Tsoory, Vouimba, et al., 2008; Yee, Plassmann, & Fuchs, 2011).

The majority of animal studies concerning the effects of early life stress on the function of the immune system have focused on exposure to stress during the perinatal and/or pre-weaning periods, which correspond to the stage of infancy in humans. In the present study, we focus on the effects of exposure to stress during juvenility. While there is strong support to indicate that exposure to juvenile stress (JVS) can induce behavioral metaplasticity, data concerning the long-term effects of exposure to stress during this sensitive developmental period on the immune system is still lacking.

Over the years, numerous clinical and experimental studies have shown that psychological stress can impact the immune function in adulthood (Dhabhar, 2014; Godbout & Glaser, 2006; Gouin, Glaser,

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Table 1
Experiment layout. SD male rats (22 PND) were housed in the animal facility for five days of acclimation. The JVS group and the JVS + EE group were exposed to the JVS protocol (27–29 PND). At 30 PND the EE group and the JVS + EE group were transferred to the EE cages and remained there for the entire duration of the experiment. All four groups were assessed behaviorally (OF and EPM) in adulthood. Immune system functionality was challenged using carrageenan (1.5 mg/kg, i.p.). Rats were decapitated 16 h post peritonitis induction. Blood and peritoneal fluid were taken for immunological tests.

Group/PND	22	27–29	30	59	60	61
1. JVS	Arrival + acclimation	JVS	–	OF + EPM	Inflammation	Tissue collection
2. JVS + EE	Arrival + acclimation	JVS	Transferred to EE cages	OF + EPM	Inflammation	Tissue collection
3. EE	Arrival + acclimation	–	Transferred to EE cages	OF + EPM	Inflammation	Tissue collection
4. Control	Arrival + acclimation	–	–	OF + EPM	Inflammation	Tissue collection

Malarkey, Beversdorf, & Kiecolt-Glaser, 2012; Padgett & Glaser, 2003). Interestingly, some of these studies show that stress occurring at an early stage of life can cause long-lasting changes in the immune function, as observed both in humans and animals (Avitsur et al., 2015; Coelho, Viola, Walss-Bass, Brietzke, & Grassi-Oliveira, 2014). For example, clinical studies show that individuals who have suffered a history of early-life stress display long lasting alterations in pro-inflammatory mediators (Carpenter et al., 2010; Danese et al., 2008; Lopes et al., 2012). Similarly, neonatal stress increases pro-inflammatory cytokine expression and viral replication in influenza virus-infected adult mice (Avitsur, Hunzeker, & Sheridan, 2006). While several studies indicate that the immune system contributes to metaplasticity-like changes (Grau et al., 2014; Huie et al., 2012), the metaplasticity-like effects on the adult immune functions resulting from exposure to JVS have yet to be determined.

Previous studies have shown that exposure to an enriched environment (EE) regulates excitability, synaptic transmission, and long term potentiation (LTP) in the dentate gyrus of rats, indicating the potential role of EE in metaplasticity effects (Irvine, Logan, Eckert, & Abraham, 2006). Exposure to EE conditions can also ameliorate stress-induced behavioral alterations and significant EE effects have been reported following early life stress. For instance, Cui and colleagues (Cui et al., 2006) demonstrated that, in young adult rats, exposure to EE helped to completely overcome the effects of early life stress, which included impaired spatial learning, increased depressive-like behavior, and impaired hippocampal LTP. In addition, EE has been also shown to reverse most of the effects of exposure to JVS not only on the behavioral level, but also on the endocrine and biochemical levels (Ilin & Richter-Levin, 2009).

Studies concerning the effects of exposure to EE on the immune system also support the beneficial aspect of this behavioral manipulation. For instance, it has been demonstrated that environmental enrichment has positive effects not only on behavioral parameters, but also on the activity of natural killer (NK) cells in mice (Benaroya-Milshtein et al., 2004). In addition, rats exposed to EE have been shown to be less vulnerable to the negative effects of repeated immune challenges (Mlynarik, Johansson, & Jezova, 2004), and exposure to EE proved to have a corrective effect on impaired spatial and contextual memory in interleukin-1 (IL-1) signaling deficient mice (Goshen et al., 2009). In another study, following an immune challenge, rats exposed to EE displayed reduced expression of pro-inflammatory cytokines within the hippocampus compared to rats in standard housing conditions (Jurgens & Johnson, 2012; Williamson, Chao, & Bilbo, 2012). Chabry and colleagues have shown that EE favors an anti-inflammatory activation state by blocking gene induction of pro-inflammatory cytokines in brain-sorted microglia. In addition, they have shown that EE housing significantly increases the percentage of macrophages expressing IL-4Rα and chemokine receptor type 2 (CCR2), similarly to what has been observed in microglia (Chabry et al., 2015).

In the current study, we set out to investigate the metaplasticity-like effects of exposure to JVS on behavior and on the immune system function, specifically focusing on trafficking of monocytes and macrophages during inflammation and their relevant chemokines and receptors, while also examining the ability of EE housing conditions following exposure to JVS to alter these effects.

2. Materials and methods

2.1. Animal subjects

Male Sprague-Dawley (SD) rats (Envigo Laboratories, Israel) were habituated in the Brain and Behavior Research Laboratory facilities for five days before starting the experiment. The rats were maintained for the entire duration of the experiment on a 12/12 h light-dark cycle (lights on at 07:00–19:00), room temperature of 22 ± 2 °C, with four rats per cage (35 × 60 × 18 cm^3) on sawdust bedding (replaced once a week), with water and solid food pellets provided *ad libitum*. Weekly body weight measurements took place throughout the entire experiment period. All procedures and tests were approved by the Institutional Animal Care and Use Committee and adhered to the guidelines of the US Institute of Laboratory Animal Research Guide for the Care and Use of Laboratory Animals.

2.2. Experimental design

In order to evaluate the long term metaplasticity effects of the exposure to JVS and the influence of EE housing conditions, rats were assessed behaviorally for anxiety in adulthood (59 PND) using the elevated plus maze (EPM) and open field (OF) exploration test (as described in Sections 2.5.1 and 2.5.2). Immune system functionality was challenged one day after the behavioral assessments using intra-peritoneal (i.p.) injection of carrageenan (1.5 mg/kg). Rats were decapitated 16 h post peritonitis induction, since preliminary experiments indicated that in this model of induced peritonitis, inflammation reaches its peak 16 h post injection (data not shown). Blood and peritoneal fluid were taken for immunological tests (as described in Section 2.6) (see Table 1).

2.3. Juvenile stress protocol

The procedure was comprised of three sequential days of exposure to different stressors (as discussed in Horovitz, Tsoory, Hall, Jacobson-Pick, & Richter-Levin, 2012); a different stress protocol was used each day, at approximately midday (12:00 PM). Day 1 (27 PND), forced swim: 10 min forced swim in an opaque circular water tank (diameter: 0.5 m; height: 0.5 m; water depth: 0.4 m), water temperature 22 ± 2 °C. Day 2 (28 PND), elevated platform: 70 cm above floor level, located in the middle of a small closet-like room. Rats were subjected to three 30 min trials with inter-trial interval of 60 min in the home cage. Day 3 (29 PND), restraint: rats were placed in a metal mesh restraining box that prevents forward–backward movement and limits side-to-side mobility. Rats remained in the restraining box for 2 h (Tsoory & Richter-Levin, 2006).

Protocols were applied simultaneously to all rats in the same cage, in order to prevent isolation. Following the completion of the stress procedure, rats returned to their home cages and were not handled until reaching sexual maturity, at the age of ~60 PND, except for weekly weighing and cage sawdust bedding maintenance.